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DIVERSITY AND ANTIBIOTIC RESISTANCE OF ENTEROBACTERIA ISOLATED FROM BROILERS IN A POULTRY FARM OF PERM KRAI: A 14-YEAR STUDY

M.V. KUZNETSOVA, E.V. AFANASIEVSKAYA, M.O. POKATILOVA, A.A. KRUGLOVA, E.S. GOROVITZ

Wagner Perm State Medical University, 26, ul. Petropavlovskaya, Perm, 614990 Russia, e-mail mar@iegm.ru (✉ corresponding author), lizavika@mail.ru, pokatilovamery@gmail.ru, aakry@mail.ru, eduard.Gorovitz@mail.ru
ORCID:
Kuznetsova M.V. orcid.org/0000-0003-2448-4823
Afanasyevskaya E.V. orcid.org/0000-0002-3498-6459
Pokatilova M.O. orcid.org/0000-0001-5564-248X
Kruglova A.A. orcid.org/0000-0003-4556-1162
Gorovitz E.S. orcid.org/0000-0003-4320-8672
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Abstract

Sanitary and anti-epizootic measures in poultry industry should restrict spread and circulation of antibiotic-resistant bacteria, including pathogens which are the causative agents of epidemic diseases. This paper is our first report on prevalence and antibiotic resistance of enterobacteria in a commercial poultry flock population during a 14-year period with an assessment of the effectiveness of the most common fluoroquinolone- and colistin-containing veterinary medicines. Our goal was to compare species diversity and the antibiotic resistance of enterobacterial strains isolated from cross Ross 308 broiler chickens (*Gallus gallus*) in a large poultry complex (JSC PRODO Perm Poultry Farm, Perm Krai). Dead embryos and trachea, lungs, heart, liver, spleen, femur and tibia bones collected from slaughtered broilers were the pathomaterial (995 samples in 2004-2009, and 991 samples in 2010-2017). The organs of healthy broilers served as a control. In special experiments, we assessed the effectiveness of veterinary drugs used against certain pathogens. It was found that the frequency of *Enterobacteriaceae* family members remained stably high during the whole observation and significantly exceeded that of gram-positive microorganisms ($p < 0.00001$). In 2010-2017, bacterial contamination was higher in heart ($p < 0.05$) and spleen ($p < 0.01$), while the rate of infected dead embryos averaged 25 % and was lower ($p < 0.05$) compared to 2004-2009. *Escherichia coli* dominated, and *Proteus mirabilis* was a subdominant species. The prevalence of avian pathogenic *E. coli* (APEC) in the microbial community did not change, *Proteus* isolates ($p < 0.0001$) increased, and salmonella decreased ($p < 0.05$), the prevalence of other enterobacteria did not differ significantly. The prevalence of antibiotic-resistant *E. coli* was multidirectional: the ciprofloxacin-resistant strains increased from 47.4 to 75.9 % ($p < 0.0005$), whereas the amikacin-resistant strains decreased from 32.8 to 16.4 % ($p < 0.0001$). It was shown that Coliflox (NEC Agrovetszashchita SP, LLC, Russia) which contains maximum dosage of colistin and enrofloxacin is the most active among four studied colistin-containing medicines. Further down medicines are Vitrocil (Interchemie werken De Adelaar BV, Netherlands), which contains 10 times less colistin and 2 times less enrofloxacin, Pulmosol® (VIK — Animal Health LLC, Belarus) and Aquaprim (SP Veterinaria, SA, Spain) with 1.1 million and 400,000 IU colistin, respectively. Pulmosol® and Aquaprim, lacking of fluoroquinolones, showed the least activity (37.5 and 35.7 % of resistant *E. coli*, respectively, and 50.0 and 37.5 % of resistant *Proteus* spp.). Thus, combined veterinary medicines containing enrofloxacin had the highest activity against enterobacteria, but the effectiveness of these drugs has decreased in recent years. The long-term use of fluoroquinolones as dietary additives to prevent infections among poultry seems to be a risk factor leading to the selection of resistant strains.

Keywords: poultry farms, monitoring, enterobacteria, *Escherichia coli*, antibiotic resistance

Bacterial infections are second only to viral infections and cause significant damage to poultry production worldwide, as they can cause up to 35% mortality [1]. This problem is particularly acute due to the increase in food-borne diseases [2, 3]. Ensuring effective protection of poultry from infectious pathology has been and remains one of the main tasks of veterinary medicine [4, 5].

The conditions of large poultry farms with high planting densities cause circulation among birds and people of different microorganisms, creating the risk of disease outbreaks. Animals can be a source of pathogenic and conditionally pathogenic bacteria — *Salmonella enterica*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* [6-9]. As a rule, gram-negative bacteria cause an acute course of diseases. The same chemicals are used for their treatment and prevention for a long time. This leads to the spread of antibiotic-resistant strains of microorganisms, primarily enterobacteria, which become the main depot of antibiotic-resistance genes. *E. coli* is already almost completely insensitive to tetracyclines and nalidixic acid [10]. Koga et al. [11] revealed a significant number of *E. coli* isolates producing extended-spectrum β-lactamases (*ESBL*). In salmonella isolated from birds, multiple resistances to antibacterial drugs is often found [8, 12, 13]. The fluoroquinolone-resistant species of enterobacteria circulating in poultry enterprises have been described [14, 15].

It is known that bacteria infectivity, species and antibiotic resistance of the livestock vary considerably from farm to farm. Control of bacterial pathogens is necessary to assess the epizootic situation in poultry farms and to select active antibacterial agents. In addition, since most of the antibiotics used in the poultry industry are also used in medicine, it is of interest to analyze the prevalence and resistance profiles of enterobacteria found in poultry farms.

This study presents for the first time in the comparative aspect the data of the long-term monitoring of the prevalence and antibiotic resistance of enterobacteria with the complex evaluation of the effectiveness of the main veterinary drugs containing fluoroquinolones and colistins. It has been established that the isolation rate of *Enterobacteriaceae* bacteria from bird organs was much higher than that of gram-positive microorganisms, and the number of generalized forms of colibacillosis increased. Enrofloxacin-containing preparations were the most active against enterobacteria, but as a result of long-term use for antibacterial therapy in industrial poultry production facilities, their effectiveness was reduced.

The aim of the work was to study species diversity and carry out a comparative assessment of antibiotic resistance of enterobacterial strains isolated from broiler chickens in a large poultry complex.

Techniques. Pathological material (trachea, lungs, heart, liver, spleen, bone tissue − femoral and tibial bones) were collected from broiler chickens (*Gallus gallus*) (Ross 308 cross; PRODO Perm Poultry Farm JSC, Perm Region) after slaughter and from died (not hatched) chickens. A total of 995 samples were tested in 2004-2009 [16] and 991 samples in 2010-2017. The control was the organs of healthy birds. The biomaterial was selected monthly. The age of the birds varied from 1 to 34 days and averaged 19.7±4.4 days. In addition, water samples from the drinking system were analyzed (as a likely source of birds' infection).

In bacteriological analysis, the biomaterial collected aseptically was used for plating by Gold's method on MacConkey medium (Sigma, USA) and blood agar. The isolated pure cultures were identified to a species using the diagnostic systems Entero-Test16 and NefermTest24 (Lachema, Czech Republic). The sensitivity of isolated strains to antibacterial drugs was determined by the disc-diffusion method according to MG (methodological guidelines) 4.2.1890-04 using Muller-Hinton agar (Merk, USA) and commercial discs manufactured by NITsF LLC (St. Petersburg, Russia) or HiMedia Laboratories Pvt. Ltd. (India) containing the following antibiotics (μg): ampicillin — 10, amoxicillin-clavulanate — 20/10, cefotaxime — 5, meropenem — 10, gentamicin — 10, amikacin — 30, ciprofloxacin — 5, levofloxacin — 5, chloramphenicol — 30, tetracycline — 30, nalidixic acid — 30 and furazolidone — 50. The *Escherichia coli* reference strain ATCC 25922 served as an internal control.

In a series of special experiments, the effectiveness of veterinary drugs used in the specified farm was determined in relation to some pathogens of bacterial diseases of birds. The following drugs were used: Aquaprim (SP Veterinaria, S.A., Spain), Vitrocil (Interchemie werken De Adelaar B.V., Netherlands), Koliflox (LLC NEC Agrovetzashchita S-P., Russia), Pulmosol® (LLC VIC – Animal Health, Belarus), Triflon (LLC Vitavet, Russia), Ciprovet (LLC NEC Agrovetzashchita S-P., Russia), Enroflon (LLC VIC — Animal Health, Belarus). All preparations are included in the Register of Medicines and Feed Additives for Animals approved for use in Russia, belong to hazard class III or IV according to GOST 12.1.007-76 (except for Triflon, hazard class II) and are assigned to broiler chickens with therapeutic and preventive purposes. Their effectiveness was evaluated by serial dilutions with regard of the working dose of the drug as per the manufacturer's instructions.

Statistical processing of the obtained data was carried out using Microsoft Excel 2016 and STATISTICA 10 software (StatSoft, Inc., USA). The indicators are presented as the arithmetic mean and its error ($M\pm$SEM). To identify statistically significant differences between the two independent samples, χ^2 or χ^2 with the correction of Yates (Frank Yates) was determined.

Results. During our observation, the isolation of gram-negative microorganisms from the organs of broiler chickens after forced slaughter remained high (Fig. 1). In 2012-2014, there was a slight decrease in the isolation rate, but since 2015 it again exceeded 50% of all the samples studied. Despite the fact that in recent years there has also been an increase in poultry infection with gram-positive microorganisms, in 2010-2017 the samples with gram-negative bacteria (59.0%) significantly exceeded the same indicator for gram-positive bacteria (14.4%) ($p < 0.00001$). In general, in 2004-2009 and 2010-2017, the average infection rates of poultry with bacterial microflora did not differ significantly (56.9±2.2% and 59.0±13.3%, respectively). The infection of addled eggs at the second stage of the study was 25% and was lower than that found in 2004-2009 ($p = 0.0462$).

In water from the drinking system, enterobacteria were isolated in more than 80% of cases in both observation periods.

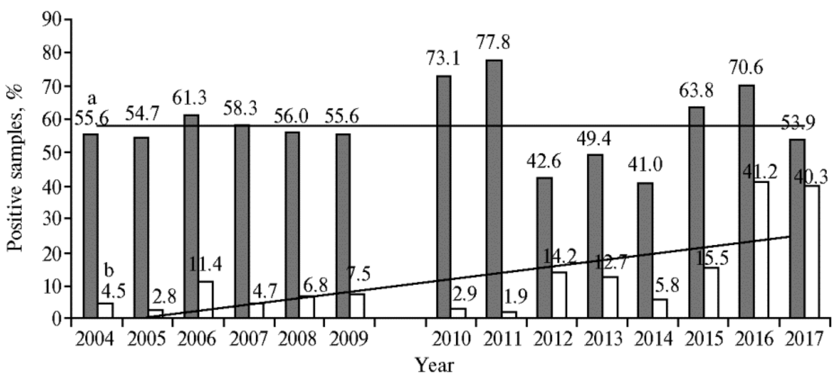

Fig. 1. Microbial contamination of the internal organs of broiler chickens (*Gallus gallus*) of the Ross 308 cross with gram-negative (a) **and gram-positive** (b) **bacteria in 2004-2009 [16] and in 2010-2017 (**PRODO Perm Poultry Farm JSC, Perm Region). The straight lines reflect trends in representation for each group of microorganisms.

Infection with gram-negative bacteria of trachea, lungs, heart, liver, spleen, and broiler bone reached, 31.2%; 73.1%; 64.3%; 62.5%; 65.7% and 32.3%, respectively (Table 1). Bacteria were isolated less often from bone tissue than from parenchymal organs and lungs ($p < 0.0001$). There were no statistically significant differences between infection of the heart, liver, and spleen. The trachea was infected less than the lungs ($p < 0.0005$), which turned out to be the most bacterized of all the studied material. Compared to 2004-2009, bacterial contamination of the heart ($p < 0.05$) and spleen ($p < 0.01$) were significantly higher.

1. Contamination by gram-negative bacteria of the internal organs of cross Ross 308 dead embryos, broiler chickens (*Gallus gallus*) and water from the drinking system in different years (PRODO Perm Poultry Farm JSC, Perm Region)

Source	No.	Organ, biotope	2004-2009 [16]		2010-2017	
			n	positive, *n* (%)	*n*	positive, *n* (%)
Broilers	1	Trachea	–	–	16	5 (31.2 %)
	2	Lungs	6	5 (83.3 %)	104	76 (73.1 %) $p_{1-2} < 0.0005$
	3	Heart	264	143 (54.2 %)	244	157 (64.3 %)
	4	Liver	276	186 (67.4 %) $p_{3-4} < 0.01$	307	192 (62.5 %)
	5	Spleen	180	90 (50 %) $p_{4-5} < 0.01$	134	88 (65.7 %)
	6	Bone	–	–	155	50 (32.3 %) $p_{2-6} < 0.0001$ $p_{3-6} < 0.0001$ $p_{4-6} < 0.0001$ $p_{5-6} < 0.0001$
	7	Kidneys	–	–	12	0
	8	Ovary	110	64 (58.2 %)	–	–
	9	Other organs	10	6 (60 %)	3	2 (66.7 %)
	10	All organs	846	494 (58.4 %)	975	570 (58.5 %)
Embryos	11		149	75 (50 %)	16	4 (25 %) $p_{10-11} < 0.05$
Drink system	12	Water	142	124 (87.3 %)	15	13 (86.6 %)

N o t e. Dashes indicate a lack of data for the specified period. The subscripts at p are the numbers of the compared samples.

As expected, the infection of the birds by *E. coli* prevailed throughout the investigation, averaging 43.4±7.7% and 53.8±12.4%, respectively, for 2004-2009 and 2010-2017 (see Fig. 2, A). Among enterobacteria, *Escherichia* comprised 63.3% in 2004-2009, 69.6% in the subsequent period, that is, did not significantly differ (see Fig. 2, B). *Proteus* spp. (*Proteus mirabilis*, *P. vulgaris*, etc.) varied from 7.4% in 2006 to 45.7% in 2016 and averaged 22.8% over the past 8 years, which was almost 2 times more than in 2004-2009 (12.2%, $p < 0.0001$). The Salmonella infection rate was high (26.6%) in 2009 with a significant proportion of isolates of embryonic origin. In 2010-2017, they were significantly less than in the previous period. In general, for 2010-2017, the species diversity of enterobacteria isolated from poultry after forced slaughter did not change significantly compared to 2004-2009, while we found a significant increase in the counts of the *Proteus* genus representatives, a tendency to an increase in *Escherichia* infection and, which is essential, a decrease in the frequency of salmonella, including in embryonic material.

Since *E. coli* and *Proteus* spp. were the dominant bacteria throughout the observation period, we assessed the antibiotic sensitivity of these microorganisms using the disk diffusion method. A total of 511 strains of *Escherichia* and 172 strains of *Proteus* were analyzed. When choosing the tested antibacterial drugs, both the duration of their use in veterinary practice and the species characteristics of the isolates were taken into account. The strains of *E. coli* resistant or conditionally resistant to ampicillin and cefotaxime among the strains isolated in 2010-2017 comprised 74.1% and 17.8%, respectively (Table 2). In the first stage of the study (in 2004-2009), all bacteria were sensitive to meropenem. Amikacin resistance ranged from 5.9% to 34.9% and averaged 16.4% over 8 years, which is statistically significantly lower than in the previous period ($p < 0.0001$). Similar trends were found for gentamicin: in 2010, the gentamicin-resistant *E. coli* strains reached almost 60.0%, and in 2017 only 6.2%. Bacterial resistance to ciprofloxacin, on the contrary, statistically significantly increased to 75.9% in the second period of the study ($p < 0.0005$). The number of strains of *E. coli* resistant to levofloxacin was lower, but they still accounted for about half of the cultures. Similar trends were observed for *Proteus*: a significant portion of strains resistant to ampicillin (42.5%) and fluoroquinolones, the ciprofloxacin (36.8%), and norfloxacin (33.4%), were detected, while resistance to gentamicin and amikacin declined (20.2% and 2.1%, respectively). Almost all cultures of the studied enterobacteria were insensitive to furazolidone, tetracycline, and nalidixic acid.

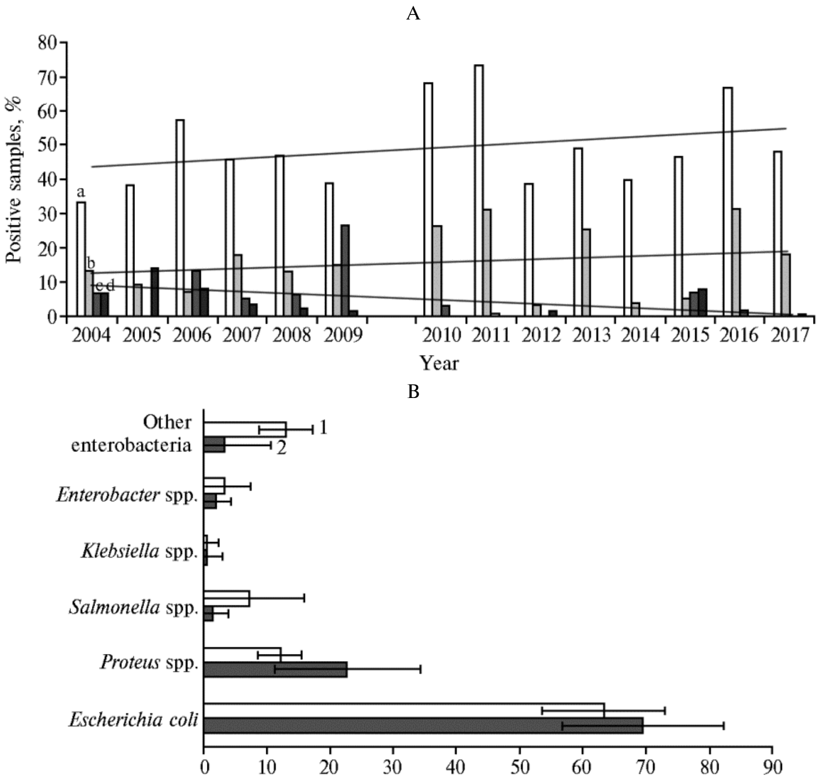

Fig. 2. Contamination by *Enterobacteriaceae* of Ross 308 (A) **cross broiler chickens *(Gallus gallus*) and specific weight** (%) of ***Enterobacteriaceae*** (B) **in different years:** a — *Escherichia coli*, b — *Proteus* spp., c — *Salmonella* spp., d — другие энтеробактерии; 1 — 2004-2009 [16], 2 — 2010-2017. *Proteus* spp. comprised *P. mirabilis*, *P. vulgaris*; *Salmonella* spp.comprised *Salmonella* serovar Enteritidis, *S. tiphimurium*, *S. arizonae* etc.; *Klebsiella* spp. comprised *K. pneumoniae*, *K. oxytoca*; *Enterobacter* spp. comprised *E. sakazakii*, *E. aerogenes*, *E. cloacae*; enterobacteria comprised *Escherichia vulneris*, *Citrobacter freundii*, *Morganella morganii* (PRODO Perm Poultry Farm JSC, Perm Region). The straight lines reflect trends in representation for each group of microorganisms.

2. Antibiotic resistance of *Escherichia coli* isolates (absolute number of resistant isolates/%) **from the internal organs of Ross 308 cross broiler chickens (*Gallus gallus*) in different years (**PRODO Perm Poultry Farm JSC, Perm Region)

Ampicillin	2004-2009 (*n* = 390) [16]	2010-2017 годы (*n* = 511)
Amoksiklav	321/82.3	379/74.1
Cefotaxime	181/46.4	–
Meropenem	87/22.3	91/17.8
Gentamicin	0	0
Amikacin	212/48.9	54/10.6*
Ciprofloxacin	128/32.8	84/16.4*

Table 2 continued

Levofloxacin	185/47.4	388/75.9*
Chloramphenicol	–	221/43.2
Tetracycline	208/53.3	–
Nalidixic acid	385/98.7	443/86.7
Furazolidone	–	459/89.8
Ampicillin	359/2.1	476/93.1

N o t e. Dashes indicate a lack of data for the specified period.
* Differences with the previous period are statistically significant at $p < 0.05$.

3. Resistance of *Escherichia coli* and *Proteus* spp. isolates from the internal organs of Ross 308 cross broiler chickens (*Gallus gallus*) to some drugs (PRODO Perm Poultry Farm JSC, Perm Region)

Drug (working dose), active substance	Amount of active substance	Strians, *n*/%					
		E. coli		*Proteus* spp.		enterobacteria	
		total	resistant	total	resistant	total	resistant
Aquaprim (1 ml/l):							
lincomycin	50 mg						
sulfamethoxol	200 mg						
trimethoprim	20 mg						
colistin	400,000 IU	16	6/37.5	6	3/50.0	22	9/40.9
Vitrocil (0.5 ml/l):							
enrofloxacin	50 mg						
colistin	600,000 IU	24	8/33.3	11	2/18.2	35	10/28.6
Coliflox (1 ml/l):							
enrofloxacin	100 mg						
colistin	7 million IU	12	1/8.3	5	1/20.0	17	2/11.8
Pulmosol® (150 g/l):							
kitasamycin	52.5 mg						
colistin	1.1 million IU	42	15/35.7	16	6/37.5	58	21/36.2
Triflon (1 ml/l):							
enrofloxacin	100 mg						
trimethoprim	50 mg	5	0	2	1/50.0	7	1/14.3
Ciprovet (1 g/l):							
ciprofloxacin	100 mg	39	6/15.3	18	4/22.2	57	10/17.5
Enroflon (1 g/l):							
enrofloxacin	100 mg	53	8/15.1	25	3/12.0	78	11/14.1

N o t e. Dilutions (working doses) is given according to the manufacturer's instructions. Data for *Salmonella* of Enteritidis serovar are not presented.

In a special series of experiments, we assessed the effectiveness of seven veterinary drugs against the main pathogens of bacterial infection of birds, *E. coli*, *Proteus* spp. and *Salmonella* of Enteritidis serovar. Of the four studied colistin-containing drugs, Coliflox, represented by a combination of colistin at the maximum dose and enrofloxacin, had the highest activity (Table 3). Vitrocil which contained 10 times less colistin and 2 times enrofloxacin, Pulmosol® and Aquaprim (the dose of colistin 1.1 million IU and 400,000 IU respectively), followed in descending order. The last two preparations that do not have fluoroquinolones in their composition showed the lowest activity: 37.5% and 35.7% of *E. coli* cultures, 50% and 37.5% of *Proteus* spp. cultures were resistant to them.

Mono-preparations containing fluoroquinolones suppressed the growth of more than 80% of all tested enterobacteria. Enroflon was not statistically significant, but more effective than Ciprovet, primarily due to the effect on *Proteus* strains. At the same time, Triflon, in which trimethoprim is also present, was less active against *Proteus* than Enroflon, but this may be due to a smaller sample of cultures. All cultures of *Salmonella* of Enteritidis serovar ($n = 3$) were sensitive to the studied combined veterinary drugs in a working dose. It should be noted that in most cases, bacterial growth was inhibited when diluting drugs in a dose less than 2 times lower than the working one, for example, when testing Coliflox (data not shown). In this case, the growth of both cultures resistant to Coliflox in the working dose was inhibited by the drug added in a double dose. Similar data were obtained for the remaining tested drugs. Enterobacteria were also resistant to increased concentration of the drug, and their number increased in dynamics: for Aquaprim there were 7 of 9 such cultures (77.7%; 1 culture in 2015 and 6 in 2017), for Vitrocil — 6 out of 10 (60%; all cultures were identified in 2017), for Pulmosol® — 11 out of 21 (52.4%; 3 cultures in 2015 and 8 cultures in 2017), for Ciprovet — 5 out of 11 resistant cultures (45.4%; all cultures highlighted in 2017).

As per annual medical statistics, bacteria of the genera *Salmonella*, *Escherichia*, and *Campylobacter* are the most common causative agents of anthropozoonoses that are associated with poultry products. The industrial maintenance of the poultry serves as the powerful stress by itself modulating reactions of the congenital and induced immunity, reducing the resistance of birds even to own microbiota that creates preconditions for the influence of bacteria on an organism and fast distribution of pathogens of diseases. In recent years, the circulation of opportunistic and pathogenic microorganisms that can cause human food poisoning has increased in poultry farms [17-19]. The need to study the dynamics of the spread of antibiotic resistance in representatives of the *Enterobacteriaceae* family is not in doubt.

It is known that in specialized poultry farms of Russia, the death of birds from coli infection remains high for many years [20]. Thus, according to the All-Russian Scientific Research Veterinary Institute of Poultry (St. Petersburg), the specific rate of *E. coli*, isolated in recent years from various species of birds in several regions of Russia, is at least 40% [21]. Microbiological monitoring at 11 poultry farms in Western Siberia established the dominance of *E. coli* among gram-negative microorganisms (48%). Representatives of the *Citrobacter* and *Proteus* genera were isolated only in 13% and 6% of cases [22]. In several poultry farms in Ukraine, *Escherichia* amounted to 30.8% in the microbiota of dead birds [23].

The most important problem for all commercial poultry is systemic coli infection, as well as respiratory diseases such as aerosacculitis [20, 24]. In a multicenter monitoring study conducted by Kalinin et al. [25], it was shown that in bird respiratory syndrome, a gram-negative flora was isolated in most cases (*E. coli* — 36.5%, *P. vulgaris* — 11.7%, *P. aeruginosa* — 5.8%, *Salmonella* of Enteritidis serovar — 3.9%). In this study we revealed the *E. coli* infection to prevail, and the rate of *Escherichia* among enterobacteria was stably higher than 60%. A predominant proportion of *E. coli* was observed in all organs and tissues. *E. coli* was isolated from the heart in 80.3% of cases, which coincides with the fact that fibrinous pericarditis is the most typical sign of colibacteriosis [21].

During the incubation and rearing of birds, the counts of microorganisms and diversity of microflora increases, reaching a maximum during hatching [22]. Indeed, we found the embryos to be less infected than broiler chickens. Earlier, during a 6-year observation of the development of birds in dynamics from days 1 to days 43-45, we showed that infection is determined by the age of the bird [16]. If in 1-day-old chickens the internal organs were practically sterile, then by day 40 their bacterial contamination reached 78.3%.

The problem of antimicrobial resistance over the past 20 years has gained particular importance in connection with the intensification of agriculture. The 68th World Health Assembly adopted the Global Plan of Action to Reduce Antimicrobial Resistance (resolution WHA68.7 dated May 26, 2015). A comprehensive and intersectoral approach, which provides for a system to control the circulation of antibiotics, is proposed for industries where they are actively used, including for agriculture.

Our results indicate that over the years, the antibiotic resistance of

E. coli strains at the Perm Territory's poultry farm to some drugs has varied widely, while to other drugs has remained relatively stable. It should be noted that since 2011, antibiotics have not been added to feed. Thus, the resistance of the strains to ampicillin, tetracycline, furazolidone and nalidixic acid remained almost unchanged, and resistance to these drugs can be considered as a fixed sign, which, apparently, is associated with the prolonged use of these chemotherapeutic agents in the poultry farm. Nevertheless, it was possible to identify the dynamics of changes in the resistance of strains to aminoglycosides and fluoroquinolones. In the first case, the number of resistant strains isolated from birds decreased statistically significantly, which is probably due to the complete cessation of the use of these drugs since 2013, which contributes to the elimination of such strains from the ecosystem. The results obtained indicate that antibiotic rotation can be effective in controlling strain resistance.

Resistance to fluoroquinolones, on the contrary, has increased significantly, which may be associated with prolonged use of monocomponent and complex fluoroquinolone-containing preparations (Enroksil, Ciprovet, Enroflon, Hydroquinocol, Coliflox, Quinoprim, Triflon). The active substance of most of them is enrofloxacin, which is partially metabolized in the liver to ciprofloxacin and inhibits bacterial DNA gyrase and topoisomerase IV. At the same time, fluoroquinolone-containing preparations showed the greatest antimicrobial activity (as assessed by the serial dilution method), although their effectiveness began to gradually decrease.

Our data on the effectiveness of a number of drugs are consistent with studies by other authors. In the article of Miles et al. [10], it was found that 82.4% strains of *E. coli* isolated from chickens were resistant to tetracycline, 85.3% to nalidixic acid, and only 8.8% to ciprofloxacin. Moreover, almost 30% strains were stable and about 40% were moderately resistant to enrofloxacin. In another work, the sensitivity tests of enterobacteria to 15 drugs showed that aminoglycosides, neomycin, and gentamicin were effective, and most of the *Escherichia* were resistant to other drugs, including the fluoroquinolone series (enroflox, enroflon) [19]. Thorsteinsdottir et al. [26] found that enrofloxacin-resistant *E. coli* strains were found in 33.6% of cases when screening for isolates from broiler chickens and in 52% when screening for food products (broiler meat). In Iran, among 318 APEC strains (avian pathogenic *E. coli*) isolated from broiler chickens with generalized coli infection, 37.7% were resistant to enrofloxacin, but only 7.5% were resistant to ciprofloxacin and 5.7% to gentamicin [27]. According to the verification of veterinary drugs in the framework of microbiological monitoring at poultry farms in Ukraine, a high bactericidal activity of fluoroquinolones, the enroxil and sarafloxacin, was revealed [22]. An observation at federal poultry processing plants in Canada showed that in chicken herds all *E. coli* strains were sensitive to amicacin, ciprofloxacin and enrofloxacin, but it should be noted that these data were obtained in 2003-2004 [28].

Consequently, the trend towards an increase in the number of antibiotic-resistant strains of bacteria is characteristic of different countries. A connection has been shown between the use of specific drugs in poultry enterprises and the resistance of circulating bacteria to them [28]. Most reports indicate almost complete resistance of APEC to tetracycline, mediated by the *tetB* and *tetD* efflux-genes which are often associated with conjugative plasmids [10]. At the same time, sensitivity to aminoglycosides remains rather high. The data regarding quinolones and fluoroquinolones are contradictory: in a large part of the studies, high resistance to nalidixic acid is described, but the rate of strains resistant to ciprofloxacin and enrofloxacin can vary significantly. It has been proven that isolates resistant to fluoroquinolones can circulate even in the absence of further drug exposure, and therefore some countries have abandoned the use of fluoroquinolones in poultry farming [29]. The issue of the prospect of using colistin (the polymyxin group) is being actively discussed: first, because of the increasing bacterial resistance due to the spread of the *mcr-1* gene the treatment of colibacteriosis and salmonellosis in animals may be ineffective, and second, in medicine it is necessary to maintain the effectiveness of the drugs used for combating pathogens with multidrug resistance [30]. Providing systematic monitoring of the spread of antimicrobial resistance has become part of the program "The Strategy for Preventing the Distribution of Antimicrobial Resistance in the Russian Federation until 2030" (Decree of the Government of the Russian Federation No. 2045-r dated October 25, 2017).

Thus, this paper represents results of monitoring enterobacteria in the microbiota of broiler chickens in a large poultry farm in the Perm Territory. It was found that the rate of enterobacteria isolated from poultry organs during all the observation periods remained stably high and significantly exceeded the corresponding indicator for gram-positive microorganisms. In 2004-2009 and 2010-2017, the average contamination of poultry organs with gram-negative bacteria did not statistically significantly differ (56.9±2.2% and 59.0 ± 13.3%, respectively). Lungs, heart, liver, broiler spleen were contaminated in more than 50% of cases, trachea and bone tissue in every third bird. Apparently, the bacterial contamination of various organs of industrial poultry is associated with the generalization of the infectious process typical of broilers, which is due to reduced immunological resistance because of the peculiarities of housing conditions. The proportion of APEC strains (avian pathogenic *Escherichia coli*) in the total microbial composition did not change, while the *Proteus* strains increased and salmonella decreased, the frequency of other representatives did not differ significantly. The revealed trends in the prevalence of antibiotic-resistant *E. coli* were multidirectional. The number of ciprofloxacin-resistant strains increased, and the rate of amikacin-resistant decreased. Antibiotic resistance of bacteria (bacterial factor) and immunological suppression (macroorganism factor) contributed to the generalization of coli infection: indicators of bacterial contamination of the heart and spleen were significantly higher compared to the previous period. Of the studied veterinary antibacterial drugs, Coliflox, the combination of colistin and enrofloxacin, has the greatest activity against enterobacteria circulating in the enterprise. Despite the fact that the effectiveness of fluoroquinolone-containing preparations has been decreasing in recent years, the rejection of their use and replacement with others is apparently inadvisable today. The data obtained confirm the need for systematic monitoring bacterial pathogens in poultry enterprises and their antibiotic resistance, which will allow adequate and effective use of antimicrobials in veterinary practice.

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